Developmental Pyrethroid Exposure Reproduces Features of Attention-Deficit Hyperactivity Disorder

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Scope of the Problem:

Attention-deficit hyperactivity disorder (ADHD) is estimated to affect 8-12% of school-aged children worldwide (1). Although genetic factors account for a large percentage of ADHD cases, an estimated 20-40% of cases do not appear to have a primary genetic etiology, suggesting that environmental factors may contribute to the disease. Studies have identified dopamine transporter (DAT) polymorphisms and elevated striatal expression of the DAT in ADHD patients, suggesting alterations in DAT levels may contribute to ADHD (2). Since environmental factors, such as pesticides, have been shown to alter DAT expression (3,4), we hypothesized that in utero exposure to pesticides may contribute to the incidence or severity of ADHD.

Methods:

Animal Treatments: Female C57 Bl/6J mice (8-10 weeks old) were administered 0, 0.3, 1, or 3 mg/kg deltamethrin orally in peanut butter every three days for two weeks throughout gestation and lactation. Pups were weaned on postnatal day (PND) 21 and experiments conducted on pups 6-12 weeks of age.

Dopaminergic Neurochemistry: Western blots for the dopamine transporter (DAT) were performed as described previously (Richardson and Miller, 2004). Blots were stripped and reprobed for alpha-tubulin to ensure equal protein loading among samples. mRNA levels were determined by real-time PCR in midbrain samples.

Locomotor Activity: Mice were allowed to acclimate to the open-field for 30 min and then activity monitored for an additional 60 or 90 min. Drug treatment was conducted at the 30 min time point and then activity was monitored for an additional 90 min. The location of the animal and total distance traveled was tracked with the Noldus Ethovision system.

Y-Maze Testing: Mice were introduced to the Y-maze and allowed to freely explore the maze for 8 minutes. The location of the animal was tracked with the Noldus Ethovision system. Tapes were reviewed by a blind observer and the % alternation was calculated in overlapping triplet units. A same arm repeat was defined as an animal leaving a single arm and immediately returning to that same arm.

Statistical Analysis: Each litter was treated as an individual experimental unit. Neurochemical data were analyzed by Student's t-test or by one or two-way ANOVA followed by means separation by the Dunnett's Test where appropriate. Significance is reported at the $p \leq 0.05$ (*), $p \leq 0.01$ (**), or $p \leq 0.001$ (***).

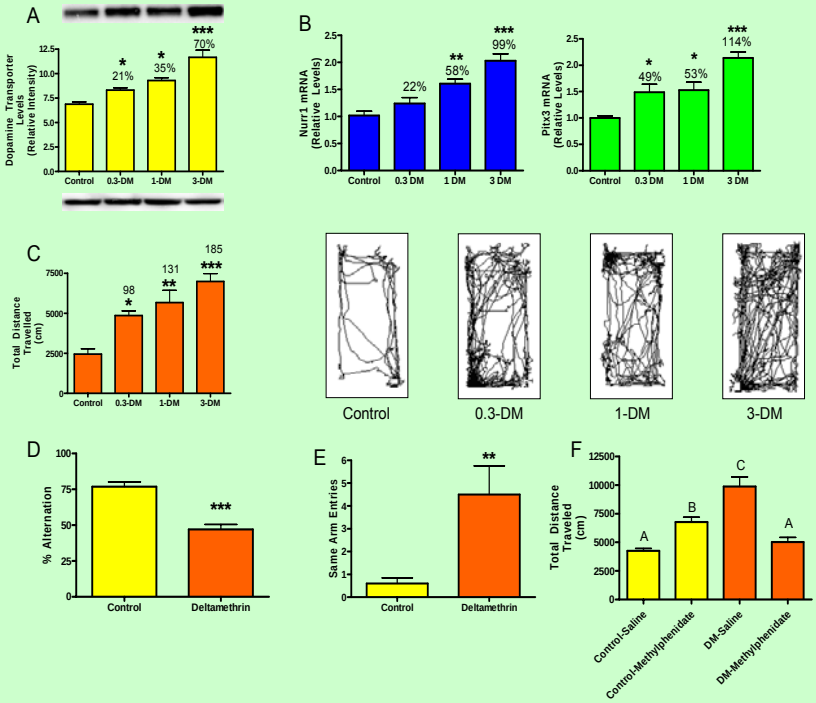
Results:

To model in utero exposures, we exposed pregnant mice to the commonly used pyrethroid pesticide deltamethrin (DM) at doses of 0, 0.3, 1, or 3 mg/kg every three days throughout gestation and lactation. These doses are well below the developmental no-observable adverse effect level (NOAEL) for this pesticide, which is considered the most sensitive indicator of toxicity by the Environmental Protection Agency. At 6 weeks of age, which is roughly equivalent to the adolescent period in humans, DAT protein levels were elevated in the striatum of the male deltamethrin offspring by 21%, 35%, and 70% (Fig 1A), similar to the range observed in imaging studies with ADHD patients (2). Female deltamethrin offspring were much less affected with DAT increases of only 13%, 22%, and 31%.

DAT expression during development is under coordinate control of the nuclear transcription factors Nurr1 and Pitx3 (5), thus, we measured the mRNA levels of these transcription factors. Nurr1 (22%, 58%, 99%; Fig 1B) and Pitx3 (49%, 53%, and 114%) mRNA levels were significantly increased in the midbrain of the male offspring.

Locomotor activity was increased in male offspring by 98%, 131%, and 185% over an additional 90 min period, which is illustrated by their locomotor track tracings (Fig 1C). Female deltamethrin offspring did not exhibit significant hyperactivity, which is reminiscent of the lower rates of ADHD diagnosis in girls. The hyperactivity was accompanied by behavioral deficits in working memory (reduced alternation behavior) and attention (increased same-arm entries) as determined by Y-maze testing (6). Deltamethrin male offspring exhibited significantly decreased alternation behavior (77% for controls and 47% for deltamethrin mice) and increased same-arm entries (0.6 for control offspring and 4.6 for deltamethrin offspring; Fig.1D, E).

Finally, we determined whether the commonly used therapeutic agent for ADHD, methylphenidate (Ritalin®) could effectively reduce hyperactivity. Administration of 0.1 mg/kg methylphenidate (i.p.), which is in the range administered to children (approximately 0.25 mg/kg orally), slightly increased locomotor activity in control offspring, whereas it reduced locomotor activity to control levels in the deltamethrin (3 mg/kg) male offspring (Fig. 1F).



Discussion:

- Developmental exposure of mice to levels of deltamethrin below the developmental NOAEL produces long-term alterations of the dopamine system resulting in hyperactivity and deficits in attention and working memory.
- The up-regulation of DAT appears to be related to increased expression of Nurr1 and Pitx3, two nuclear transcription factors required for the proper development and maintenance of the dopamine system.
- These data suggest that developmental exposure of mice to deltamethrin produces a neurochemical and behavioral phenotype similar to that observed in children with ADHD.
- Pyrethroid pesticides are often considered the "safe" alternative to more toxic pesticides, such as the organophosphates. Given the dramatic effects of this study and the documented exposure of pregnant women to pyrethroids (7), it may be prudent to evaluate pyrethroid exposure as a potential risk factor for ADHD.

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